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Neil R. Blacklow, M.D.

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This project is assessing the etiology and establishing the rapid diagnosis of human viral gastroenteritis. Close attention is being given to the development and utilization of immunoassays to detect various etiologic agents, with the preparation and use of monoclonal antibody reagents where possible. Following the successful development of rapid diagnostic assays, the medical and epidemiological importance of various etiologic agents is being ascertained. During the contract year for this report, we have succeeded in developing and evaluating an enzyme immunoassay (EIA) test for the direct detection of astrovirus (small, round diarrhea viruses) in human diarrheal stool samples. This test depends on the use of our previously developed monoclonal antibodies that react with a group antigen shared by all known astroviruses. The astrovirus EIA test has detected 31 of 33 stool specimens positive for astrovirus by immune electron microscopy (IEM) (sensitivity of 94%) and has reacted with 2 of 57 gastroenteritis stool specimens negative for astrovirus by IEM (specificity of 96%). It has detected all 5 known astrovirus serotypes in stool speci-

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19. Abstract

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ETIOLOGY AND RAPID DIAGNOSIS OF HUMAN VIRAL GASTROENTERITIS

Annual Report

Neil R. Blacklow, M.D.

May 1, 1989

(For the period 1 May 1988 - 30 April 1989)

Supported by

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SUMMARY

This project is assessing the etiology and establishing the rapid diagnosis of human viral gastroenteritis. Close attention is being given to the development and utilization of immunoassays to detect various etiologic agents, with the preparation and use of monoclonal antibody reagents where possible. Following the successful development of rapid diagnostic assays, the medical and epidemiological importance of various etiologic agents is being ascertained. During the contract year for this report, we have succeeded in developing and evaluating an enzyme immunoassay (EIA) test for the direct detection of astrovirus (small, round diarrhea viruses) in human diarrheal stool samples. This test depends on the use of our previously developed monoclonal antibodies that react with a group antigen shared by all known astroviruses. The astrovirus EIA test has detected 31 of 33 stool specimens positive for astrovirus by immune electron microscopy (IEM) (sensitivity of 94%) and has reacted with 2 of 57 gastroenteritis stool specimens negative for astrovirus by IEM (specificity of 96%). It has detected all 5 known astrovirus serotypes in stool specimens, and has not reacted with 90 stools from patients with diarrhea due to other small round gastroenteritis viruses (Norwalk, Snow Mountain, Hawaii, and calicivirus). This EIA test to detect astroviruses in stool specimens will now permit us for the first time to assess the role of these viruses in acute gastroenteritis by performing extensive epidemiological studies. Quantitative techniques to be utilized in epidemiological and biological studies of human astroviruses are also being developed through virus plaquing and neutralization techniques. Using these methods, we have established that the Marin County agent of diarrhea is an astrovirus serotype 5, that we have been able to detect directly in stool samples. Efforts continue to try to cultivate in vitro other small gastroenteritis viruses, specifically Norwalk virus and calicivirus, using techniques that we have successfully used to grow astroviruses, as well as using culture media with supplements such as small intestinal secretions. We have also employed our previously developed monoclonal antibody based EIA for the detection of enteric adenovirus types 40 and 41 in human stools in studies of their medical importance in overseas populations. We have found that small percentages (one to three per cent) of pediatric diarrhea have been caused by enteric adenoviruses in both outpatient Thai children and hospitalized Australian Aborigine children in whom rotavirus was a more common pathogen. This relative infrequency of enteric adenovirus infection contrasts with a higher incidence reported for children in temperate climates. Ongoing collaborative epidemiological studies have continued to be carried out with the U.S. military in overseas populations and in soldiers traveling overseas, with particular emphasis on use of the rapid immunodiagnostic procedures that we have developed for viral gastroenteritis agents.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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BACKGROUND INFORMATION ON VIRAL GASTROENTERITIS

Acute viral gastroenteritis is an extremely common illness that affects all age groups and occurs in both epidemic and endemic forms (1). It is second in frequency only to the common cold among illnesses affecting United States families under epidemiological surveillance. It is also responsible for some of the common travelers' diarrhea encountered in Latin America, Africa, and Asia. The illness varies in its clinical presentation, but in general it begins with an explosive onset, and consists of varying disabling combinations of diarrhea, nausea, vomiting, low grade fever, abdominal cramps, headache, anorexia, myalgia, and malaise. It can be severe, indeed fatal, in the elderly, infant, debilitated, or malnourished patient.

Viral gastroenteritis occurs primarily in two epidemiologically distinct clinical forms (1). One entity is characteristically epidemic and it responsible for family and community-wide outbreaks of gastroenteritis among older children and adults. In recent years, one agent, Norwalk virus, has been shown to be responsible for about 40 percent of these disease outbreaks in the United States. Other Norwalk-like viruses have also been discovered such as Hawaii agent and Snow Mountain agent, and although they have not been well studied epidemiologically, they are likely to be responsible for many more epidemic cases of this illness.

The second clinical entity is usually sporadic, occasionally epidemic, and occurs predominantly in infants and young children (1). However, as noted below it can occur in adults. This form of illness typically produces severe diarrhea that commonly lasts for five to eight days and is usually accompanied by fever and vomiting. Rotavirus is responsible for nearly one half of the cases of this clinical entity requiring hospitalization. Although the major target of rotavirus is the very young, it can produce surprisingly severe clinical disease in adults (1,2).

Breakthroughs in determining the medical importance of Norwalk virus and rotavirus occurred primarily because of the development of immunoassay techniques to recognize these viruses in stool samples and to measure antibodies to them in infected individuals. For Norwalk virus, these assays are currently available in only a few research laboratories (3,4) including that of the principal investigator. This is because the procedure requires use of precious limited human volunteer materials (stools and sera). The assay has more recently been made more efficient in detecting Norwalk virus antigen in stools through the use of an enzyme-linked immunoassay (EIA) instead of a radioimmunoassay (RIA) (5). Together with our collaborators, our use of these immunoassays has shown a major role for this virus in producing in the U.S. clam and oyster associated gastroenteritis, as well as some cases of travelers' diarrhea in Mexico and Thailand (6-8).

As for rotavirus, use of immunoassay techniques to detect the virus is now common and is employed routinely in many clinical diagnostic laboratories (9). More recently, a monoclonal antibody based EIA that we developed for detection of rotavirus (10) has been shown to be more sensitive and specific than polyclonal antibody tests and has eliminated specificity problems with stool samples from young infants. We have used rotavirus immunoassays to establish the role of rotavirus in several nations around the world,

including travelers' diarrhea experienced by U.S. military populations overseas (7,11-15).

The roles of other enteric viruses in gastroenteritis are poorly understood, and because of the medical importance of infectious diarrhea, there is clearly a major need to establish the significance of different viruses that may be involved. Comparative studies on their occurrence, however, have been infrequent and usually limited to electron microscopy (15-17). The major obstacle in evaluating the relative importance of the non-rotavirus and non-Norwalk virus enteric viruses as causative agents of gastroenteritis has been the lack of convenient methods for their diagnosis. In addition, for appropriate treatment and control measures to be initiated, rapid as well as convenient methods are required, but have been also unavailable for most of these gastroenteritis viruses. Further, many of these viruses are difficult to cultivate or have not been cultivated in cell culture, which has inhibited characterization studies.

Among these agents, the evidence currently seems strongest that "enteric" adenoviruses are medically important pathogens like rotavirus and Norwalk virus. These adenoviruses differ from the well characterized conventional serotypes of adenoviruses which are propagated in standard tissue cultures and are not commonly associated with gastroenteritis. The "enteric" adenoviruses are recognized by electron microscopy in stools and cultivatable inefficiently in an adenovirus transformed cell line, Graham 293 (18). Two "enteric" serotypes (types 40 and 41) have been identified and in a limited number of studies performed to date, have been highly associated with gastroenteritis in infants and young children and much less commonly found in asymptomatic children (1,19). The potential role of enteric adenoviruses in travelers' diarrhea or in disease in adults has been little studied. Convenient and specific immunoassays to detect enteric adenoviruses have been only recently developed and now permit an understanding of their epidemiology as has already occurred with the use of immunoassays to study rotavirus and Norwalk virus. Two years ago, we prepared monoclonal antibodies specific for adenovirus types 40 and 41. These antibodies were characterized and used in an EIA format to detect the enteric adenoviruses in known positive diarrheal stool specimens with 95 to 98 per cent sensitivity and specificity (20,21). During 1988, we reported that two percent of acute diarrheal episodes among Thai children were due to enteric adenoviruses using the EIA procedure (22). We are now using our enteric adenovirus monoclonal antibody EIA to assess the epidemiology of this infection in other populations.

Caliciviruses have also been associated with diarrheal disease in humans (1,23). These agents are currently detected mostly by electron microscopy and more convenient assays for their detection are needed so that their epidemiology can be studied. Norwalk virus possesses a single structural protein, characteristic of a calicivirus (24), and the two agents are of similar size and general shape (albeit, differing somewhat in virion surface structure). Thus, the possibility of relatedness between these two enteric viruses exists and was studied by us two years ago. We demonstrated that antigenic characteristics are shared between calicivirus and Norwalk virus based on our detection of seroconversions to Norwalk virus in patients experiencing gastroenteritis due to a strain of calicivirus (25,26). These two agents, therefore, may belong to the same family of viruses, as also may Snow

Mountain agent for which we have also found seroconversions to Norwalk virus in some affected patients (27). These serological cross-reactions demonstrate the need for convenient viral antigen specific detection methods for calicivirus such as we previously developed for Norwalk virus.

Astroviruses, like caliciviruses, are small (27-35nm in diameter) and have been identified by electron microscopy in the stools of some patients with gastroenteritis (1,28). Astroviruses have been reported to be cultivable in cell culture (29,30). However, simple diagnostic procedures have not been developed. Thus, the extent of the role of astroviruses in human diarrheal disease is not known. During 1988, we published our confirmation of in vitro cultivation of 4 of 5 serotypes of human astrovirus (31). This then permitted us to purify sufficient viral antigen to prepare monoclonal antibodies reactive against a common antigen shared by multiple astrovirus serotypes (31). This offered the practical possibility for developing immunoassays to assess the medical importance of astroviruses in human viral gastroenteritis. Such an EIA test for the detection of astroviruses in human stools has been developed by us during the current contract year.

ANTIGEN DETECTION WITH MONOCLONAL ANTIBODIES FOR THE DIAGNOSIS OF ASTROVIRUS GASTROENTERITIS

During the current (1988-1989) contract year, we have succeeded in developing and evaluating an enzyme immunoassay (EIA) test for the detection of astroviruses in human diarrheal stool samples. Critical to this test is the use of the astrovirus group-specific monoclonal antibodies that we have prepared and described in Annual Report dated May 1, 1988 as well as published during 1988 in the Journal of Infectious Diseases (31). These monoclonal antibodies react with all 5 known astrovirus serotypes by immunofluorescence, indicating that the astroviruses share a group antigen. Our initial studies also revealed that the monoclonal antibodies reacted with 4 of the 5 serotypes by EIA, but not with cell culture grown astrovirus type 1. However, during the current contract year, we have been able to demonstrate reactivity with type 1 astrovirus by using higher titer virus preparations in the EIA test. Thus, we established that the EIA test using monoclonal antibodies detects all five known human astrovirus serotypes, cultivated in cell culture.

During the first half of 1989, we have evaluated the astrovirus EIA procedure in the testing of acute diarrheal stool samples derived from patients with and without astrovirus gastroenteritis. Astrovirus-positive stool specimens were kindly provided to us by Dr. David Cubitt (London, England) and were demonstrated to contain the virus by immune electron microscopy (IEM). We also evaluated diarrheal stool samples that were negative for astrovirus by IEM and for other detectable viral and bacterial agents by culture, IEM, direct electron microscopy and immunoassays for adenovirus, Norwalk virus, and rotavirus. The results are shown in the accompanying Table 1.

Table 1. Comparison of astrovirus EIA and IEM for the detection of astrovirus in stool samples of patients with gastroenteritis

Astrovirus EIA Reactivity	Astrovirus IEM Reactivity	
	Positive	Negative
Positive	31*	2
Negative	2	55

* Numbers of stools tested

As shown in the table, 31 of the 33 specimens known to be astrovirus-positive by IEM were detected by the EIA test for a sensitivity of 94%. Of the 57 additional gastroenteritis stool specimens negative for astroviruses by IEM, two were positive in the EIA for a specificity of 96%.

In further testing, we have evaluated the astrovirus EIA procedure for its sensitivity in detecting the different astrovirus serotypes in stool samples. For this testing, we had available 16 stools for which the astrovirus serotype had already been determined. The results are shown in Table 2.

Table 2. Sensitivity of astrovirus EIA for stools containing different astrovirus serotypes

Astrovirus Serotype (Determined by IEM)	Number of Samples Tested	Mean A ⁴⁹⁰ + SD
1	6	0.49 + 0.29
2	2	0.82 + 0.52
3	1	0.72
4	5	0.49 + 0.24
5	2	1.389, 0.257 ^a

a. One sample was diluted 1:10, the second 1:100.

It is clear from Table 2 that the astrovirus EIA test is also group-reactive in the testing of the five known human astrovirus serotypes in stool samples.

We have also tested a battery of diarrheal stool samples known to be positive for small round gastroenteritis viruses other than astroviruses. These included stools containing calicivirus, Hawaii virus, Snow Mountain virus, and Norwalk virus (70, 9, 4 and 7 samples, respectively). All were negative in the astrovirus EIA.

Our data indicate that we have developed an EIA test using astrovirus-specific monoclonal antibodies that now permits a rapid and specific diagnosis of astrovirus gastroenteritis. The data are being prepared for publication. The EIA test now provides a means for epidemiological studies to be undertaken. Such studies should be able to define for the first time the extent of astrovirus involvement in gastroenteritis, which to date has been difficult to assess due to lack of convenient detection and identification methods.

PLAQUE QUANTITATION AND VIRUS NEUTRALIZATION ASSAYS FOR HUMAN ASTROVIRUSES

We are endeavoring to develop quantitative techniques to be applied to epidemiological and biological studies of the human astroviruses. These efforts have been impeded somewhat by the fact that although the astroviruses can be cultivated in cell cultures containing trypsin (31), no distinguishable cytopathic effects are observed. In last year's Annual Report, we indicated that we achieved cultivation of 4 of the 5 known astrovirus serotypes, and during the current contract year we have successfully cultivated the fifth serotype in vitro (type 3) following multiple serial passages in HEK cells. We have now attempted to develop plaque quantitation techniques for each of the 5 serotypes. We have been able to obtain well defined plaque formation in LLCMK₂ cells with astrovirus types 1, 2 and 5 under an agar overlay containing trypsin. The titers obtained with cultivated astroviruses were 3.5×10^4 , 1.2×10^4 , and 1.1×10^5 plaque-forming units per ml for astrovirus types 1, 2, and 5 respectively.

Rabbit antisera to astroviruses, determined to be serotype-specific by immunofluorescence, were tested for their ability to neutralize astrovirus types 1 and 2 by plaque reduction. Our monoclonal antibody to the astrovirus group antigen was also tested for virus neutralization capacity. The results, presented in Table 3, indicate that the rabbit antisera neutralized virus type specifically with the possible exception of type 1 neutralization by antiserum to type 5. There was no neutralization of the astroviruses tested by the monoclonal antibody. Thus, it appears from the neutralization pattern seen that antisera raised against specific astrovirus types (as determined by immunofluorescence) neutralize virus type-specifically. Studies on neutralization of astrovirus type 5 are in progress.

Table 3. Neutralization of astrovirus types 1 and 2.

Astrovirus serotype	1/Titer					Normal serum	Monoclonal antibody
	1	2	3	4	5		
1	3,200	<50	<50	<50	100	<50	<50
2	<50	6,400	<50	<50	<50	<50	<50

These data will permit quantitative techniques to be applied to epidemiological and biological studies of human astroviruses. The data were presented at the Annual Meeting of the American Society for Microbiology (May 1989) (32).

FURTHER CULTIVATION AND CHARACTERIZATION STUDIES ON THE MARIN COUNTY
STRAIN OF ASTROVIRUS

Marin County Agent (MCA) was first described in 1981 as the result of an investigation of an outbreak of gastroenteritis among patients and staff in a convalescent home (33). Examination of fecal samples from patients and from an adult volunteer by electron microscopy (EM) revealed the presence of large numbers of small round viruses 27nm in diameter. MCA had been placed within the Norwalk virus group although immune EM (IEM) and radioimmunoassay (RIA) tests indicated that it was antigenically distinct from Norwalk virus and Hawaii virus (33).

In Annual Report dated May 1, 1988, we presented data indicating that we had cultivated the MCA in cell culture from the stool of an infected volunteer and had identified it in the stool as a characteristic astrovirus by IEM. Further, the MCA in stool reacted by IEM with astrovirus type 5 antiserum. Also, acute and convalescent sera from an MCA-infected patient showed seroconversion to astrovirus type 5 by EIA and IEM, and also to astrovirus type 1 by IEM. Additional studies showed that the MCA grown in cell culture reacted by immunofluorescence with both astrovirus type 1 and 5 antisera and with our group-reactive monoclonal antibody. We concluded that the MCA can be propagated in vitro like other astroviruses and that it is serotypically an astrovirus type 5 that cross-reacts in some tests with type 1, and a report was published in The Lancet (34). During the current contact year, preliminary plaque neutralization data also indicate that the MCA is a typical astrovirus type 5.

Also, during the current contract year, we obtained a second stool sample of the MCA (from a naturally occurring case in the Marin County outbreak, provided by L. Oshiro) and have identified astrovirus in the sample by IEM (using convalescent serum from an MCA-infected volunteer). In addition, this second stool sample is positive in the EIA test for astrovirus. Thus, both of the MCA stools we possess are positive by IEM and EIA. In addition, we have shown that 2 of 6 paired sera from the original MCA outbreak (provided to us by L. Oshiro) have seroconverted by immunofluorescence to MCA. We have also inoculated HEK cells with the second stool sample of the MCA (from the MCA outbreak) in an attempt to cultivate it as we have with the first sample (from the MCA infected volunteer). We have detected no evidence of MCA replication from the second stool sample through 10 serial passages in HEK cells.

IN VITRO CULTIVATION STUDIES WITH NORWALK VIRUS AND CALICIVIRUS

We have continued our efforts to cultivate Norwalk virus and human calicivirus in vitro. These efforts have been encouraged by our success with the in vitro cultivation of the human astroviruses (31). As outlined in last year's Annual Report, we have inoculated the infectious 8FIIa strain of Norwalk virus into HEK cell cultures supplemented with small intestinal fluids derived from uninfected gnotobiotic piglets. These fluids were kindly provided by Dr. L. Saif, who was able to cultivate a previously noncultivable pig enteric calicivirus in vitro using these small intestinal supplements (35). We have serially passaged Norwalk virus 15 times with the porcine small intestinal fluids, and have failed to detect evidence of

Norwalk virus replication by EIA and immunofluorescence. In addition, we have also performed 15 serial passages of Norwalk virus-inoculated HEK cells supplemented with trypsin and centrifuged onto coverslips for immunofluorescence studies (this is the same technique successfully used for the cultivation of astroviruses). Again no evidence of Norwalk virus replication was apparent after 15 serial passages.

Currently, efforts are being initiated to try to cultivate Norwalk virus incorporating filtered human small intestinal fluids in HEK cells (these fluids were collected in unrelated studies 15 years ago and stored at -70°C) in the same manner employed for Dr. Saif's materials. In addition, DMSO is being used at a concentration of 1.5% in other sets of HEK cells inoculated with Norwalk virus (DMSO is known to enhance replication of other unrelated viruses).

In vitro cultivation studies with human calicivirus are in their early stages. HEK cells have just been inoculated with a virus particle-rich stool sample containing human calicivirus (kindly provided by Dr. D. Cubitt, London). Inoculated cells are being manipulated in several ways (as we have also done for Norwalk virus): (a) addition of small intestinal secretions from gnotobiotic swine; (b) addition of filtered human small intestinal secretions; (c) addition of trypsin to centrifuged cells; (d) use of DMSO. Evidence of calicivirus replication will be sought by IEM and immunofluorescence. It is our assumption that it is logical to perform the same cultivation studies with human caliciviruses as with Norwalk virus due to the evidence that these two agents are related in terms of their protein composition (24) and immunological characteristics (25,26).

INCIDENCE OF ENTERIC ADENOVIRUSES IN ACUTE GASTROENTERITIS AMONG OVERSEAS POPULATIONS

Our previous development of a monoclonal antibody based EIA for the detection of the enteric adenoviruses (types 40 and 41) in stool specimens (20,21) now enables us to perform epidemiological studies of their medical importance in acute diarrheal disease. Information about the EIA test and enteric adenoviruses is provided above in the "Background Information on Viral Gastroenteritis" section. Until our development of this rapid diagnostic test, the only reports of the incidence of adenoviruses types 40 and 41 in diarrheal disease have come from studies of pediatric diarrhea from countries with temperate climates, and have revealed their presence in about 6 to 8 percent of cases (36,37).

During the current contract year, we have published in the Journal of Clinical Microbiology a study on the incidence of enteric adenoviruses among children with and without diarrhea in Bangkok, Thailand (22). Some of these data were available in Annual Report dated May 1, 1988, but a full summary of the data is given below. The study was performed in collaboration with Drs. Peter Echeverria and David Taylor of the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok. To determine the incidence of adenovirus infection in a tropical climate, stools were collected from children under age 7 during a 1-year period at an outpatient clinic in Bangkok, Thailand. Stools from 1,114 children with gastroenteritis and from 947 children without gastroenteritis were tested. Each stool was tested for adenovirus group

antigen and for specific enteric adenovirus types (Ad40 and Ad41) by monoclonal antibody enzyme immunoassays. We found that 4.4% (49 of 1,114) of children with gastroenteritis and 1.8% (17 of 947) of children without gastroenteritis were positive for adenovirus group antigen. In tests for specific enteric adenovirus types, 2.0% (22 of 1,114) of the tests were positive in children with gastroenteritis and 0.6% (6 of 947) were positive in children without gastroenteritis. There was a significant correlation ($P < 0.02$) of gastroenteritis with nonenteric adenovirus types (27 of 1,114) as well as with specific enteric adenovirus types ($P < 0.01$). By comparison, 19.7% of children with gastroenteritis and 0.7% of those without gastroenteritis were positive for rotavirus infection. In the adenovirus-infected children with gastroenteritis, there were coinfections with rotavirus only in those with nonenteric adenovirus infection (7 of 27 children). There were no significant differences in the association of bacterial or parasitic infections with either enteric or nonenteric adenovirus infections in either group of children studied. These data demonstrate that Ad40 and Ad41 are causes of gastroenteritis in this population, but among the spectrum of diarrheal etiologies, they may be proportionately less important than they are in countries with temperate climates.

During the current contract year, we have initiated an additional study of the role of the enteric adenoviruses in pediatric diarrhea overseas. This study has been performed in the temperate climate of Australia, where the incidence of infectious diseases overall is known to be much higher in Aboriginal than other Australian children. With the collaboration of Dr. M. John Albert (Alice Springs Hospital, Alice Springs, Australia), we have had the unique opportunity to determine the incidence of enteric viruses in Australian Aboriginal children. Stool samples from 1,069 children < 5 years old hospitalized in Alice Springs for diarrhea were collected from March 1988 to February 1989. Each stool was tested for group A rotaviruses, for adenovirus group antigen, and for specific enteric adenovirus types 40 and 41 by the monoclonal antibody EIA's that we have previously developed. There were 54/1069 (5%) samples positive for rotavirus, with a sharp peak occurrence in the winter months. Of the 1,069 samples, 130 (12%) were positive for adenovirus group antigen. The incidence of adenovirus was somewhat higher in the winter months, but virus was detected in stools collected throughout the period studied. There were 13/1069 (1.2%) samples positive for adenovirus 40 and adenovirus 41, and 12 of these were adenovirus 40. Most (92%) of the enteric adenoviruses were detected in specimens collected in late spring-early summer. Our data are somewhat unexpected in that they show, despite the increased incidence of infectious diseases in general among Aboriginal children, that both rotavirus and enteric adenovirus may be proportionately less important in Aborigines as causes of gastroenteritis than they are in many populations.

FURTHER STUDIES ON THE PROPAGATION OF ENTERIC ADENOVIRUSES IN CELL CULTURES

During the current contract year, we have published in the Journal of Clinical Microbiology a study on the isolation and propagation of enteric adenoviruses in HEp-2 cells (38). This study was performed because during the course of our efforts undertaken to evaluate our monoclonal antibody based EIA for enteric adenovirus diagnosis (21), we uncovered some unusual cell growth characteristics of the enteric adenoviruses (types 40 and 41). By way of background information, the enteric adenoviruses were originally

described as viruses which could be visualized in stools of patients with gastroenteritis by electron microscopy (EM) but could not be cultivated in cell cultures generally used in diagnostic virology laboratories for isolation of respiratory adenoviruses. Subsequently, it was found that the enteric types could be cultivated in Graham 293 cells, an adenovirus type 5-transformed human embryonic kidney (HEK) cell line (18). Based on these observations, it became axiomatic that detection of adenoviruses in stools by EM, and failure to isolate them in cell lines known to support growth of other adenovirus types, was presumptive evidence for the enteric types. Conversely, it was assumed in epidemiological studies on adenoviruses that virus replication in HEp-2 or other conventional cell lines could be used to designate adenovirus isolates as non-enteric (39).

In the course of our studies evaluating our monoclonal antibody based EIA for enteric adenovirus diagnosis, it appeared that some of the enteric types could be isolated in Hep-2 cells as well as in Graham 293 cells. We therefore evaluated 82 stool samples available to us from children with gastroenteritis in Canada, England and Thailand, which had been shown to contain adenovirus by EIA and/or EM. Samples were tested for primary isolation in three cell lines obtained from American Type Culture Collection, namely, Graham 293, HEp-2 and HeLa cells. It was found that 73 of the 82 isolates typed as adenovirus 40 or adenovirus 41 by monoclonal antibody EIA and by analysis of Sma I, Hind III, and Bam HI endonuclease digests, and of these 73, 30 (41%) could be isolated in HEp-2 cells (4+ CPE). This included 43% (9/21) of those typed as adenovirus 40 and 40% (21/52) of those typed as adenovirus 41. Only two of the 73 gave 4+ CPE in HeLa cells. There appeared to be a 1+ CPE that failed to develop further in HeLa cells with 19 of 73 enteric adenoviruses, whereas all but one of the non-enteric types tested developed 4+ CPE. Based on these results, the growth characteristics of adenovirus in HEp-2 cell cultures, commonly used to distinguish enteric from non-enteric adenoviruses types are not valid in either diagnosis or epidemiological studies. HeLa cells, however, were more useful for distinguishing adenovirus isolates as enteric or non-enteric types by growth characteristics alone. For the samples studied, use of growth characteristics in HEp-2 cells would result in underestimating the incidence of enteric adenoviruses in gastroenteritis. The data comparing Graham 293 and HEp-2 cells were published in 1988 (38), and subsequently the data with HeLa cells were presented at the 28th Annual ICAAC meeting (40).

COLLABORATIVE EPIDEMIOLOGICAL STUDIES WITH THE MILITARY

We have performed several collaborative studies with U.S. military scientists during the current contract year on the role of viral agents in gastroenteritis.

One study has been performed with Dr. Peter Echeverria (AFRIMS, Thailand) as part of a Cobra Gold '88 travelers' diarrhea study. Soldiers traveling from Hawaii to Thailand and concurrently being studied for malaria prevention by weekly mefloquine or daily doxycycline were evaluated by Dr. Echeverria for the development and etiology of travelers' diarrhea. Sixty diarrheal stool samples from 30 ill individuals were studied in our laboratory by monoclonal antibody EIA's for the presence of rotavirus and enteric adenoviruses. One sample was positive for rotavirus and none for enteric

adenoviruses. Studies are currently ongoing to determine whether any soldiers seroconverted to Norwalk virus during their travel.

Another series of collaborative efforts with Dr. Echeverria are the outpatient studies of endemic longitudinally studied diarrhea among children in Bangkok. The adenovirus component (22) of these studies is highlighted in the section above entitled "Incidence of Enteric Adenoviruses in Acute Gastroenteritis Among Overseas Populations". Our laboratory also collaborated with AFRIMS in the publishing in 1989 in the Journal of Infectious Diseases (41) of a case control study of endemic diarrheal disease in this population in which rotavirus was the most common pathogen (20% of 460 ill children versus <1% of 851 control children).

An additional collaboration with Dr. Echeverria is a longitudinal ongoing study of outpatient diarrhea in Bangkok among young children less than 6 months of age in which our laboratory is currently assessing the incidence of enteric adenovirus infection. To date, 582 stool specimens from ill children have been tested, 2.9% of which contain adenoviruses (about half of which are enteric adenoviruses types 40 or 41). Of 508 stool specimens from non-diarrheic children, 2.3% contain adenoviruses (all of which are non-enteric). These studies are ongoing.

Finally, Dr. John Ticehurst of WRAIR has asked our laboratory to evaluate acute and convalescent serum pairs from humans and primates infected with enteric non-A non-B hepatitis for evidence of seroconversion to the Norwalk-calicivirus group of agents. These studies are anticipated to be performed shortly.

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PUBLICATIONS OF WORK SUPPORTED BY THIS CONTRACT

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